A GREGARINE PARASITIC IN THE DOG-FLEA, *CTENOCEPHALUS SERRATICEPS*

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The excuse for describing this parasite which inhabits the alimentary tract of the dog-flea must be in the fact that much work is now being done with fleas generally, and some confusion may be saved to others, working at these insects, if some details of its various phases are related.

This parasite was found, in varying numbers, in the fleas of two fox-terrier dogs, mother and son, which had lived in Port Said nearly all their lives. Its stages follow the well-known gregarine type frequently found in Nematodes and Culicids. It is a Cephalin showing a well-marked cycle of sporogony, all the stages of which are completed within the body of the flea, its host; while, like many other gregarines, the cycle of schizogony, so far as can be found out, is wanting.

It is convenient, for the sake of description, to begin with that phase of the cycle which is first seen in the flea.

I. THE EARLY TROPHOZOITE

This stage, as in similar Gregarinidae found in the Worms, Echinoderms, and Ascidians, is the first resting phase of the parasite in the flea. It is probably caused by the direct infection of a stomach cell by a sporozoite derived from the rupture of the sporocyst, the sporozoite having been eaten by the flea larva when crawling on the dog's back. But it is possible that it may be derived from the merozoite of a cycle of schizogony which has taken place either outside the host or within the stomach of the flea-larva; up to the present time, however, no traces of such cycle have been noted.

The early trophozoite, then, is a small circular cell embedded between the pyriform epithelial cells lining the stomach of the flea. As many as twenty-five may be found in one flea situated near the proventriculus, but occasionally near the pylorus. They are frequently found in pairs. These cells contain large refractile granules, and a readily staining nucleus. The whole cell stains before the stomach cells when the organ containing it is placed upon agar having an aniline dye in suspension. In clear specimens a slender process can be detected, by which the parasite is attached to the remains, probably, of its trophic cell that has been destroyed by it. The relation of this early form to the rostrated trophozoite next to be described is not merely conjectural, because it is only found in young infected fleas and in those containing older trophozoites which cannot be mistaken. Besides, the granules in all the early stages are very characteristic. The fact that these early forms are only found in young flea imagines makes it highly probable that this parasite is ingested by the larva, for in older fleas the more highly developed phases of sporogony only are to be found, and it is unusual to see parasites in other than contiguous stages in the same flea. The age of a flea may be roughly estimated by the degree of growth of the ova in the ovisacs in the females, and by the degree of development of the spermatozoa in the vesiculae seminales in the males. In the dog-flea the absence of spermatozoa in the spermatheca of the female is almost certain evidence of the extreme youth of the imago, because fecundation by the male takes place very soon after the metamorphosis of the female is completed; and in the male the spermatozoa within the vesiculae seminales are tied together by their heads into compact bundles very soon after the imago has hatched from the nymph.

II. THE TROPHOZOITE AND ITS DEVELOPMENT

During this phase the parasite develops a well-marked epimerite. In its growth it may reach a size equal to ten times that of a stomach epithelial cell. It is pear-shaped, and is fixed by its epimerite to the lining membrane of the stomach. The body of the cell is divided, in the early stages, by a horizontal septum into two nearly equal halves, the protomerite and the deutomerite. As the cell grows it becomes

more highly granular and consequently darker to transmitted light, and the septum less distinct. It also begins to lose its pyriform shape, and slowly reverts to its circular form. When full grown it is circular and the horizontal septum has vanished, while it is full of large refractile granules and the epimerite has disappeared. These trophozoites are usually found in pairs within the stomach and adhering to its wall.

III. THE FORMATION OF THE SPORONT

The epimerite or rostrum has completely disappeared, and the parasite is circular. The next phase seen of this particular parasite is that of the association and encystment of two sporonts. The cyst is embedded in the stomach wall, and consists of a thick fibrous capsule. The gametes appear as small granules. The analogy of similar sporonts parasitic in other members of the Arthropoda shows that they are formed in the following manner:—A male and female sporont conjugate and become encysted in their mother tissue A nuclear spindle is formed from a small portion of the nucleus of the sporont and divides, the remainder of the nucleus degenerating. The spindle then produces daughter nuclei by mitosis and they again divide, until a number of nuclei are formed which bud off the surface of the sporont. Each budded nucleus is then the primary sporoblast, and ultimately becomes the male or female gamete according to the sporont from which it was originally derived.* Whether in this instance, the male gamete becomes flagellated or not, it is impossible to say, but in the specimens examined in this stage no flagella were ever seen; however, the cephalonts are very difficult to stain, as they are surrounded with such a thick fibrous wall. The males are said to burst their way into the female half of the cephalont, and there fertilise the female gametes.

The cephalont is a very remarkable looking cell embedded in the stomach wall. It is large, frequently thirty times the size of a stomach epithelial cell, and its two granular, male and female, halves make it very characteristic and distinct. It can be rolled when the stomach is pressed under the cover-slip, but I have not succeeded in staining the gametes, as sufficient force to rupture the cephalont causes their destruction.

*I am indebted to Professor Minchin's article on the Sporozoa, in Lankester's Zoology for the description of this process.

IV. THE FORMATION OF THE OOCYST

After the fertilisation of the female gametes the male half of the cephalont degenerates, and the female portion grows until it fills the whole cell, which then separates from its attachment to the stomach wall and becomes free in the cavity. Each female gamete grows in size after fertilisation, while the fibrous cell-wall thins with the distension. The gametes, or zygotes as they must now be called, are highly refractile bodies, each about the size of a red-blood corpuscle. They are packed tightly in the fibrous capsule or sporocyst, which resembles a pomegranate that has been cut through the middle with a sharp knife. It is about fifty times the size of a stomach cell, and if the stomach wall be ruptured it may be extruded, transferred to a clean slide, burst, stained and examined. But under natural conditions it passes down to the pylorus, through which it is too large to move, and it ruptures, and the contained zygotes escape into the intestines (Malpighian tubes) under the influence of the peristaltic action.

V. THE DEVELOPMENT OF THE SPOROBLASTS

If a cyst be expressed from the stomach, crushed on a slide, and the sporoblasts stained by any method giving the Romanowski result, they will be found to be lanceolate in shape but of somewhat irregular contour. Each one has some chromatin, but this varies in amount from a minute dot to an extensive streak. The cell-wall is glistening, and appears almost chitinous, while there is some very feebly staining cytoplasm. But they have a high osmotic index, for if the intestine containing them be placed unruptured upon agar spread on a glass slide and containing polychrome methylene blue, according to the method described in the Journal of Physiology, for September 16th, 1908, by H. C. Ross, it will be noticed that the living sporoblasts accept the stain before the cells forming the lining to the tube. But even these cells accept the stain more readily than the hepatic cells, or the epithelial cells of the stomach or of the salivary glands. They are, therefore, very susceptible to external influences.

After the cyst has ruptured the sporoblasts pass into the intestine, where they undergo a still further change. They become barrel-shaped sporocysts; the contour is regular, and in the fresh

state they are yellow and roll up and down the Malpighian tube with the flow of its contents. Sometimes they are very numerous, and can be seen at once on examining the intestines.

VI. THE FORMATION OF THE SPOROZOITES

While in the intestine the barrel-shaped sporocyst gives rise, in its interior, to eight rod-shaped sporozoites. These are at first found tied together by their ends like a bundle of cigars or bananas, so that when viewed from above the sporozoite gives the appearance of eight small separate circles within a circle. Up to the present it has been impossible to separate these sporozoites or to stain them, but when the sporocyst passes into the rectum of the flea it ruptures, and the sporozoites are set free with the faeces. The ultimate destination of the sporozoites has not been traced, but they are passed with the faeces. Whether, as stated before, there is a further cycle of schizogony, or whether the sporozoites are eaten directly by the flea larva and a new cycle of sporogony started, it is not possible to say. But the flea larva is difficult to obtain, and still more difficult to dissect. During two years' work with dog fleas the number found infected with this parasite was 38 per cent.

I suggest as a name for this Gregarine, *Gregarina ctenocephali canis.*